Original Research Article

Study of biodegradation of methyl parathion in pesticide contaminated agricultural soil using bacterial species

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Abstract

The modern practices involving the use of chemical pesticides mainly organophosphorus pesticide, for crop protection and in productivity increase has led to its bioaccumulation in the environment leading to generation of various health hazards. Methyl parathion is one such organophosphorus pesticide. The objective of this study is the biodegradation of this hazardous methyl parathion. Ten bacterial species were isolated from contaminated agricultural soil, which were found to tolerate this pesticide. These tolerating bacterial species were further tested for their degradation activity using thin layer chromatography. The potential degraders were Flavobacterium sp. Staphylococcus sp. and Rhizobacterium sp. Bioremediation plays a key role in order to combat from this bioaccumulation using microorganism. This study can be utilized and further enhanced using molecular techniques to formulate the suitable innoculum for the development of effective bioremediation strategy to decontaminate harmful methyl parathion.

Key Words: Biodegradation, bioremediation, methyl parathion, organophosphorus pesticide.

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INTRODUCTION

Methyl parathion (*O,O-dimethyl O-4 nitrophenyl phosphorothioate*), an organophosphate pesticide, is an extremely toxic compound that is widely used in agriculture worldwide[1]. Methyl parathion has low persistence in the environment, with reported field half-lives of 30 days in soils. However, the continuous and excessive application allows further spread[2]. Thus, this pesticide has been classified as category-1 insecticide (extremely hazardous insecticide) by World Health Organization (WHO) and can cause lethal damages by oral, inhalation or dermal exposure. It's potential of genotoxicity, oncogenicity, reproductive toxicity, developmental toxicity, neurotoxicity and immune toxicity[3]. The mode of action of this pesticide is, it poorly inhibits acetylcholinesterase activity; however, it is metabolically activated by cytochrome P_{450} to produce oxons that are very potent inhibitors of this enzyme[4]. Initially, interaction between the pesticide and enzyme is reversible, but over time, a process called "aging" occurs, which results in the formation of a covalent bond that is much more stable. This process results from the elimination of one of the alkyl side chains of the phosphate group, leaving the hydroxyl group, which avoids regeneration of the active site of the enzyme[5]. Therefore it becomes important to keep a check on its concentration in the environment. Hence biodegradation, an intial step followed by bioremediation could be attained. The microbial ability to degrade organophosphate pesticides has proven to be a genetically preserved character that facilitates the pesticide degradation, solving problems of soil contamination. The hydrolysis of methyl parathion consisting of phosphodiester bond drastically affects its toxicity, thus being an important step for their detoxification[6]. The complete degradation of methyl parathion is conversion into *p*-nitrophenol (PNP). Soil microorganisms have ability to survive in the places that are contaminated with

pesticide. Metabolic processes of these organisms are capable of using chemical contaminants as an energy source, rendering the contaminants harmless or less toxic products[7]. Among various microorganisms, bacteria are also found to be the potential degraders of complex molecules and uses pesticides for their own metabolism and growth[8]. The main objective of this study was to isolate the potential bacterial strains from methyl parathion contaminated agricultural soil and examine their degradation activity against methyl parathion. The degradation activity was studied through Thin Layer Chromatography.

MATERIALS AND METHODS

Collection of soil sample: The rhizospheric soil samples were collected from different agricultural field having history of repeated methyl parathion application. The soil samples were collected by using auger up to a depth of 10 cm. The collected samples were air dried, ground, passed through 2 mm sieve and stored in the sealed plastic bags at room temperature. These stored samples were used for further experimentation.

Pesticide used: Commercial grade methyl parathion pesticide (2% D.P.) was used throughout the experiment. The pesticide was purchased from the local pesticide suppliers in Bhopal district of Madhya Pradesh.

Isolation and Purification of Methyl Parathion Resistant Bacteria: Total 10 morphologically distinct bacteria were isolated using enrichment culture technique for the isolation of bacterial strains capable of utilizing methyl parathion as a sole source of carbon and energy. Different concentrations ranging from 100mg/L to 1000 mg/L of methyl parathion mixed with Mineral Salt Agar medium was prepared and checked for the growth of bacteria. A single isolated colony of the pesticide resisting bacteria was picked up with the help of sterilized wire loop and was inoculated in 100mL nutrient broth and it was incubated at 37^{o}C for the characterization of isolates.

Screening of Potential Pesticide Degrading Bacteria: Bacterial isolates showing growth at higher concentration (1000mg/l) were considered and studied further. These isolates were re-streaked on Mineral Salt Agar medium (MSM) containing 20 mg/l of methyl parathion for confirmation of pesticide degradation. Then MSM plates were incubated for 2-3 days and pesticide degradation was checked through the growth of bacterial strains on MSM plates and they were reported as pesticide degrading bacterial isolates.

Characterization and Identification of Bacterial Isolates: Physical and morphological characterization of bacterial isolates were performed. Morphological characters viz. size, shape, surface, opacity, texture, elevation and pigmentation were determined by visual observation as well as by using light trans-illuminator and microscopy.

Further, identification was done by performing Gram staining and biochemical tests.

Analysis of Methyl Parathion Degradation by Thin Layer Chromatography (TLC): Pesticide extraction for chromatographic analysis- Each bacterial isolate was inoculated into 100ml of MSM broth supplemented with 200mg/l mp. After 72 hours, turbidity in the MSM broth was checked which indicated degradation of pesticide. Then 10ml of MSM broth was taken in centrifuge tube and centrifuged at 4000rpm for 20 min. 5ml of supernatant was transferred into another tube and 5ml of diethyl ether was added into it. The tube was shaken for 10 min using shaker and it was allowed to settle for 30 min. After 30 min the solvent was evaporated at room temperature and residue was obtained which was dissolved in 2 ml of ethanol, it was further taken for TLC[9].

Plate Preparation: The TLC plate was prepared by pouring aqueous suspension of silica gel and $CaSO_4$ in the ratio of 4:4.

Plate Development: Mobile phase was prepared by taking n-hexane and acetone in ratio of 9:1 and TLC chamber was saturated by it. Then in one of the TLC plate 20µl of sample (pesticide + MSM without culture) was applied and labelled it as control. 200mg/l pesticide was dissolved in ethanol and 20µl was applied into TLC plate and labelled it as reference. Similarly the different MSM cultures with pesticide were applied into the TLC plates. The plates were air dried and they all were placed in chamber containing solvent[10].

Visualizing Agent: 2% of silver nitrate was dissolved in acetone and water in ratio of 3:1. Then visualizing agent was sprayed onto all the TLC plates and they were checked for colour development[9].

OBSERVATIONS AND RESULTS

Table 1: Growth of Bacterial Isolates Resisting Methyl Parathion Containing Medium

Bacterial Isolates	Concentration of Pesticide in mg/l									
	100	200	300	400	500	600	700	800	900	1000
Iso- I	+	+	+	+	+	+	+	+	+	+
Iso-II	+	+	+	+	-	-	-	-	-	-
Iso-III	+	+	-	-	-	-	-	-	-	-
Iso-IV	+	+	+	+	-	-	-	-	-	-
Iso-V	+	+	+	+	+	+	+	+	+	+
Iso-VI	+	+	+	+	+	+	-	-	-	-
Iso-VII	+	+	+	+	+	+	+	+	+	+
Iso-VIII	+	+	+	-	-	-	-	-	-	-
Iso-IX	+	+	+	+	+	-	-	-	-	-
Iso-X	-	-	-	-	-	-	-	-	-	-

(Note: Results are the mean of three replicas)

Table 2: Physical and Morphological Test of Methyl Parathion Resisting Bacterial Isolates

Bacterial Isolates	Parameters						
	Size	Margin	Elevation	Surface	Opacity	Pigmentation	Form
Iso- I	Small	Entire	Raised	Shiny	Translucent	Red	Irregular
Iso-V	Small	Entire	Raised	Shiny	Opaque	White	Irregular
Iso-VII	Small	Undulate	Moderately Raised	Shiny	Translucent	Yellow	Irregular

Table 3: Biochemical Tests and Identification of Bacterial Isolates

Sr. No.	Biochemical Test	Bacterial Isolates		
		Iso- I	Iso- V	Iso-VII
1	Indole production Test	-	-	-
2	Methyl Red Test	-	+	+
3	Voges Proskauer Test	-	-	-
4	Citrate Utilization Test	+	+	+
5	Starch Hydrolysis Test	+	+	-
6	MacConkey Test	+	+	+
7	Gelatin Hydrolysis Test	-	+	+
8	Caesin Hydrolysis Test	+	+	-
9	Urease Hydrolysis Test	+	+	-
10.	Gram's Staining	-	-	-
11	Shape	Rod	Rod	Cocci
12	Identification	*Rhizobium sp.*	*Flavobacterium sp.*	*Staphylococcus sp.*

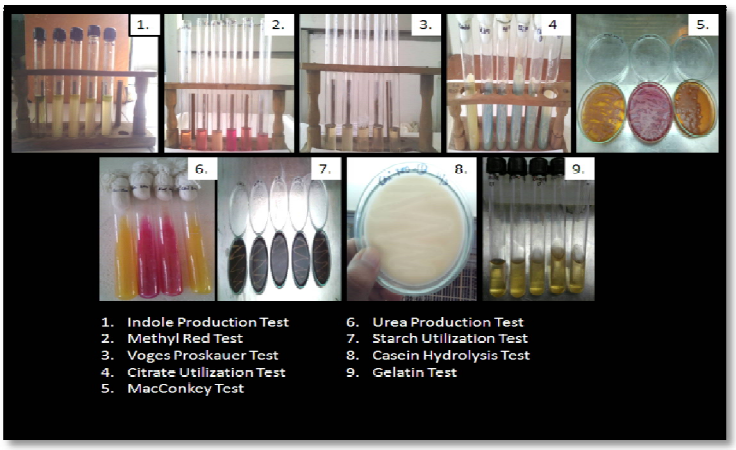

Figure 1: Results of Biochemical Test

Figure 2: Gram's Staining (From left to right- *Rhizobium sp., Flavobacterium sp., Staphylococcus sp.*)

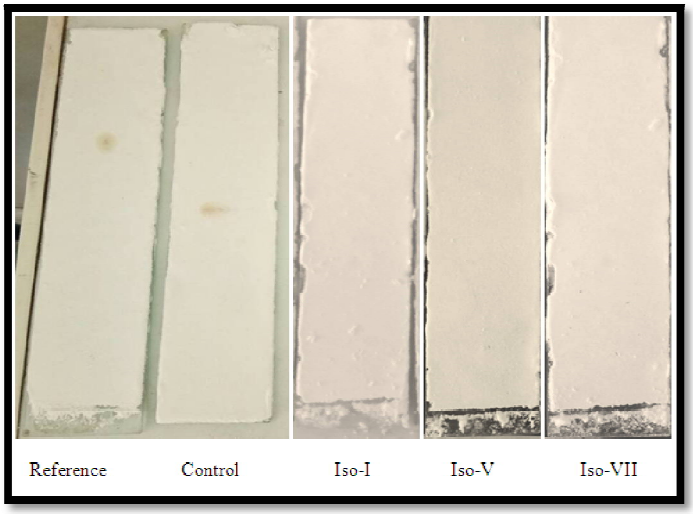

Figure 3: Analysis of Methyl Parathion Degradation through Thin Layer Chromatography

DISCUSSION

The soil samples collected from different agricultural fields showed growth of bacteria on Mineral salt agar medium (MSM) containing various concentration of methyl parathion. Out of them three bacterial isolates namely Iso-I, Iso-V and Iso-VII were found to tolerate methyl parathion and showed active growth on medium containing it. These tolerating bacterial isolates utilize and grow on mineral salt agar media (MSM). Thus, depicting their degradation activity by growing on MSM. For identification of these bacterial isolates, various physical and morphological tests were performed. Further, biochemical tests were done: Indole production Test, Methyl Red Test, Voges Proskauer Test, Citrate Utilization Test, Starch Hydrolysis Test, MacConkey Test, Gelatin Hydrolysis Test, Caesin Hydrolysis Test and Urease Production Test. Gram's staining was performed to identify bacterial isolates as gram negative and gram positive bacteria. Thus the bacterial isolates were identified as- Iso-I as *Rhizobium sp.*, Iso-V as *Flavobacterium sp.*, Iso-VII as *Staphylococcus sp.* Thin layer chromatography (TLC) was performed to detect the degradation of pesticide using cultures of these isolates. The TLC chromatographic plates of these isolates showed absence of of methyl parathion being degraded in MSM broth after 72 hours of incubation.

CONCLUSION

Isolates Iso -I as *Rhizobium sp.*, Iso -V as *Flavobacterium sp.*, Iso -VII as *Staphylococcus sp.* were marked as potential degraders. These potent isolates can be developed into immobilized culture stock which can be further used to analyze the enzymatic activity of the isolated bacteria and to develop effective treatment process for *in-situ* or *ex-situ* bioremediation. The complete biodegradation pathway for methyl parathion degradation using these degraders can be studied in future. The techniques of molecular biology and biotechnology could be exploited to improve the capabilities of the bacteria or enzymes in bioremediation systems. Present study is useful in the detoxification of methyl parathion contaminated soil and may lead to development of a suitable bioremediation technology in the near future for reclamation of pesticide contaminated soil.

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